EMBRYOLOGY.[1]

Echinoderm Eggs.—Karl Fiedler[2] endeavored to corroborate the work of Roux by experiments made at Naples upon the eggs of three species of sea urchin. At first the method used was that of Roux, piercing the egg with a needle. It was found necessary, however, to use a lancet shaped needle made by hammering out and sharpening the tip of a common needle. Later the method of shaking employed by the Hertwigs and by Boveri was, for the first time, adopted in separating the cleavage cells.

The use of the needle under the microscope was attended with a great many difficulties, increased by the large mortality amongst the few individuals that could be successfully operated upon. After the egg membrane is quickly cut the cells may be individually punctured and destroyed. Such embryos can be kept only in large vessels with algæ.

By this means it was possible to pierce a cell so that some of the contents ran out without destroying its power of cleaving, providing the nucleus remained; but in other cases where the nucleus escaped, even with but little protoplasm, the cell died. In the cases where cleavage continued the diminished cell gave rise to small progeny; thus in the four cell stage two cells were much smaller than the others; in the sixteen cell stage two of the four polar micromeres were plainly smaller than the others; in the blastula one half was less convex than the other; but later the difference seems to have been equalized.

When cells are separated by shaking, the remaining ones may change their shape, becoming more spherical where no longer in contact with other cells, but they retain their normal position much as if the other cells had not been removed. Thus when one of the first two cleavage cells was destroyed there resulted an eight celled stage that was half the normal sixteen celled stage, having half the normal number, four, of micromeres at one pole and the other cells likewise arranged as if the sixteen celled stage had been cut into two. The same was true of the half-twenty-eight cell stage. Such half embryos can be formed either by destruction of one of the first two cells or by destruction of two of the first four. The same result is produced by destroying any two of the first four cells, whether they are sister cells or first cousins.

[1]This department is edited by E. A. Andrews, Johns Hopkins University.

[2]Entwicklungsmechanische Studien. Festschrift für Nägeli, Zurich, 1891.

The first four cells are thus all alike in their possibilities as well as in appearance; not so the first eight cells, though they all look alike.

When the eight cells are separated into groups of four these give rise to quite different sets, some with eight large, others six large and two little cells, others four large and four small cells. Thus the development of the eight cell stage is largely a process of "self differentiation" of these cells and not due to the mere interaction of the cells of the group.

By destroying one of the first two cleavage cells half-blastulas even, perhaps, half-gastrulas were reared in a few cases from the single remaining cleavage cell. The half-blastulas were obtained both after piercing and removing one cell and by killing it by shaking, which left it still inside the intact membrane.

The half-blastulas showed a tendency to close in as spheres, but died first.

One interesting case suggesting Roux's "postgeneration" was seen, but lost. It consisted of a half blastula lying against a second solid hemisphere covered over by a layer of cells continuous with the half-blastula.

Electricity and Cleavage.—Wilhelm Roux,[3] seeking to determine if electrical phenomena are involved in the process of karyokinetic cell division, subjected frogs' eggs to the action of a current from three Bunsen cells. In these experiments, made in 1885[4], the eggs were placed in a glass tube surrounded by the coiled wire conveying the current.

The result was negative. In the present paper the author describes the results obtained by the use of an alternating current of 100 volts or less, used for lighting the Anatomical Institute at Innsbruck. Here again, the result as far as any connection of cleavage or cell division and the electric current is concerned, was entirely negative. When the current was not strong enough to kill the eggs they divided in the glass tube without any reference to the presence and direction of the current. The same is found to be true of the maximum continuous as well as of the alternating current.

The alternating current is also found to have no directive effect upon the entering sperm or the fusing pronuclei, factors which Roux regards as determining the first cleavage plane. The electric current has, however, a marked effect upon the egg, visible as a change in color at

[3]Bresl. ärzt. Zeitschrift, 1885, No. 6.

[4]Sitzb. Akad. Wiss. Wien., Jan., 1892.

each pole as contrasted with the equator, where such a contraction takes place that at first one might regard the equatorial belt as a cleavage furrow. These changes involve the death of the egg, and are merely a "morphological" polarization produced by the passing current and localized by its direction irrespective of any axial or polar differentiation within the egg itself.

However interesting and valuable the long series of experiments recorded in the two hundred pages of this memoir may be to the physicist and to the biologist, they have for the embryologist of the present day too little direct bearing to make it worth while reviewing them at length.

It should be mentioned, however, that the most diverse objects, frogs' eggs, gall bladders, embryos, hearts, hydras, tritons, lizard and fish embryos, chick and mammal embryos, as well as inorganic substances, such as mercury, copper, lead, etc., exhibit visible differentiation of polar and equatorial areas dependent upon the direction of passing currents. Yet this is not true of even all organic bodies experimented with.

Membranes of the Sea Urchin Egg.—Curt Herbst[5] repeated the experiment of the Hertwigs, and used in addition to chloroform, clove oil, creosote, xylol, toluol and benzole.

Eggs of the sea urchin shaken in water that had been mixed with small quantities of any one of these substances form an artificial egg-membrane just like that normally formed after the entrance of a sperm in fertilization.

This membrane, the author holds, is made by the hardening of the preexistent hyalin outer layer of the egg.

The subsequent separation of the membrane from the surface of the egg is probably due to the secretion of some jelly-like substance. The egg does not shrink away from the membrane at all.

The sperm has no direct part in the formation of the membrane, but merely acts as a stimulus to the egg. If the membrane is removed from a fertilized egg (by shaking) the presence of more sperms does not cause the formation of a new membrane. If, however, some of the above substances are used, a second membrane is formed. Two membranes, one inside the other, may be formed from eggs having one membrane, or even from those having the first membrane removed. The cause of this membrane formation is to be sought for in the egg itself.

[5]Biol. Centralblatt, Jan., 1893.

Experiments on Cleavage.[6]—Dr. Jacques Loeb, of the University of Chicago, has made a most valuable addition to our knowledge of the cleaving ovum. His experiments were simply the exposure of the eggs of sea urchins (*Arbacia*) to water containing more or less than the normal amount of sodium chloride.

The general result is stated as follows: "If we reduce the irritability of the protoplasm of the egg by reducing the amount of water contained in it, the nucleus can segment without segmentation of the protoplasm. If we increase again later the amount of water, and consequently the irritability of such an egg, the protoplasm at once divides into about as many cleavage cells as there are nuclei pre-formed. The segmentation of the protoplasm in the egg, and probably in every cell, is only the effect of a stimulus exercised as a rule by the nuclei."

The following illustration of the character of the experiment is presented by the author: Eggs taken a few minutes after impregnation were divided into four lots, one put into normal sea water, one into that concentrated by adding 2 g. Na Cl per 100 ccm., the other two into sea water concentrated by addition of 1.3 g. NaC and 1 g. Na Cl per 100 ccm. in each case.

When the eggs in normal sea water had arrived at the two celled stage none of the others had as yet begun to cleave. In the least concentrated solution the cleavage soon followed and in the more concentrated solution it followed about an hour later, but in the most concentrated solution no cleavage took place. Concentrations greater than 2 g. per 100 ccm. produced plasmolysis. The form of the cells indicates the amount of water and the intracellular pressure; thus in normal water the first two cells are nearly hemispheres, but in concentrated solutions the cells approach more and more toward a spherical shape.

Other experiments bring out the interesting point that the effect of salt is not to destroy but to suspend the cleavage phenomena. When the eggs are put back into normal water after staying some time, but not as long as twelve hours, in concentrated water, the suspended cleavage begins and goes on much as in a normally situated egg. The longer the eggs have been in the concentrated water the more numerous are the cleavage cells formed all at once when the egg is returned to normal water. An interval of about twenty minutes in the normal water must elapse before the sudden appearance of the retarded cleavage cells occurs.

[6]Journal of Morphology, vii, 1892.

The behavior of the nuclei of the eggs in concentrated sea water was observed somewhat in live eggs and in certain stained eggs which Dr. Conklin prepared for the author. It seems that the nuclei increase in numbers in the salted sea water when there are no cleavage furrows visible on the outside of the egg, but this increase is not always accompanied by a normal separation.

In the light of the conceptions of Fol and O. and R. Hertwig regarding the effects of polyspermy in producing a cleavage into many simultaneously formed cells it might have been urged that Dr. Loeb's results were due to polyspermy.

Granting, however, that the increase in nuclei takes place while the eggs are in the salted sea water such facts as the effect of this water in paralyzing the spermatozoa, as observed by the author, show that the spermatozoa cannot be connected with these peculiar cleavage phenomena.

Regarding the method of action of the salt used in these experiments we must premise that the author in previous work upon hydroids came to the conclusion that growth and regeneration in animals and plants is a function of the amount of water contained in the cells. The application of this to the present case is in the idea that increasing the concentration of the liquid about a cell decreases its irritability by removing water from it; the effect is the same quantitatively and qualitatively as would be produced by lowering the temperature.

The normal source of the stimulus which the abstraction of water is supposed to render no longer efficient to produce cleavage is considered to be the nucleus. The nature of this stimulus is unknown, but some facts lead toward the assumption that it may be a chemical one.

On the other hand the protoplasm has some influence upon the nucleus; possibly the intracellular pressure determining the form of the cell also fixes the direction of the nucleus, which will then be less defined in a mass without cell walls.